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## Claims

- 1. A process for obtaining mammalian insulin secreting cells in vitro,5 characterized in that it contains the following steps:
  - a) preparation of the mammalian pancreatic tissues from previously removed pancreata,
- b) dissociation of the pancreatic tissues obtained in step (a) into isolated pancreatic cells,
  - c) possibly the elimination of the endocrine cells from the pancreatic cells isolated in step (b),

d) induction of dedifferentiation of the cells isolated in step (b) into ductal precursor cells,

- e) induction of redifferentiation of the ductal precursor cells obtained in step (d) into insulin secreting cells.
  - 2. A process according to Claim 1, characterized in that the dissociation of the pancreatic tissues in step (b) is carried out by enzymatic digestion.
- 3. A process according to either of Claims 1 and 2, characterized in that the elimination of endocrine cells in step (c) is carried out by means of density gradient centrifugation.
- 4. A process according to any one of Claims 1 to 3, characterized in that the elimination of the endocrine cells is carried out by withdrawal of the fraction of the endocrine cells recovered in a density range between 1.027 g/L to 1.104 g/L, preferably between 1.045 g/L to 1.097 g/L.
- 5. A process according to any one of Claims 1 to 4, characterized in that
  the exocrine cells devoid of endocrine cells are recovered after centrifugation of the
  pancreatic cells isolated in step (b), in the pellet from the density gradient residue.

- 6. A process according to either of Claims 1 or 2, characterized in that the elimination of the endocrine cells is carried out by means of a cell separator.
- 7. A process according to any one of Claims 1 to 6, characterized in that the dedifferentiation of step (d) includes the following substeps:
  - i) culturing of the cells obtained in step (c) with a cell concentration between 1 x  $10^6$  and  $10 \times 10^6$  cells/mL, preferably between  $2 \times 10^6$  and  $6 \times 10^6$  cells/mL, in a culture medium containing:
  - -glucose at a concentration between 1 and 10 g/L, preferably between 2 and 5 g/L.
- -possibly serum, chosen from fetal calf serum, bovine serum or human serum, at concentrations greater than 8%, preferably between 10 and 15% final volume.
  - -a mixture of insulin, transferrin, selenium used at a concentration between 0.2 and 3%, preferably between 1.0 and 2.5%,
- -possibly factors preventing the growth of fibroblasts at a concentration between 20 and 100  $\mu$ g/mL, preferably between 30 and 60  $\mu$ g/mL,
  - -possibly antibiotics, antifungal agents,
- 25 for a duration between 4 to 9 days, preferably 5 to 7 days,
  - ii) recovery of the ductal precursor cells obtained in step (i).
- 8. A process according to one of Claims 1 to 7, characterized in that the induction of the redifferentiation of step (e) includes the following substeps:
  - i) possibly the separation of the ductal precursor cells obtained in step (d)
- ii) culturing of the ductal precursor cells obtained in step (i) at cell concentrations between 3.5 x 10<sup>5</sup> cells/25 cm<sup>2</sup> and 4 x 10<sup>6</sup> cells/25 cm<sup>2</sup>, preferably 7 x 10<sup>5</sup> cells/25 cm<sup>2</sup> to 3 x 10<sup>6</sup> cells/25 cm<sup>2</sup>, in a culture medium containing:

	-glucose at concentrations between 1 and 10 g/L, preferably between 2 and 5 g/L.
5	-possibly serum, chosen from fetal calf serum, bovine serum or human serum, at concentrations greater than 2.5%, preferably between 5 and 15% final volume.
	-possibly a mixture of insulin, transferrin, selenium at a concentration between 0.2 and 5%, preferably between 0.5 and 2%,
10	-possibly antibiotics and antifungal agents,
	-possibly in the presence of a matrix,
15	for a duration between 12 and 36 h,
	iii) withdrawal of said culture medium, and of the non-adherent cells possibly present,
20	iv) culturing of the cells obtained in step (iii) in a culture medium such as that used in step (i), possibly containing growth factors,
	for a duration between 4 and 12 days, preferably between 5 and 10 days,
25	in order to obtain insulin secreting endocrine cells, and
	v) recovery of the insulin secreting cells obtained in step (iv).
30	9. A process according to any one of Claims 1 to 8, characterized in that the separation of the ductal precursor cells obtained in substep (i) of step (e) is done with trypsin/EDTA at concentrations between 0.01 and 0.1% trypsin, preferably 0.015-0.03, and EDTA, between 0.1 and 1 mM, preferably 0.25-0.75 mM.

matrix used for culturing of the cells in substep (ii) of step (e) is chosen from collagen

type IV, 804G, collagen type I, Matrigel.

10. A process according to any one of Claims 1 to 9, characterized in that the

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- 11. A process according to any one of Claims 1 to 10, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of the pancreas of a brain dead adult human.
- 5 12. A process according to any one of Claims 1 to 10, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of a pancreas of a living patient suffering from a pancreatic pathology.
- 13. A process according to Claim 12, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of a pancreas of a living patient suffering from diabetes.
  - 14. A cell preparation which can be obtained by the process according to any one of Claims 1 to 13, characterized in that it has a cell concentration between 1 x  $10^6$  and  $10 \times 10^6$  cells/mL, preferably between 2 x  $10^6$  and  $6 \times 10^6$  cells/mL.
  - 15. Use of a cell preparation according to Claim 14 for the preparation of a pharmaceutical composition which can be used for the treatment of pancreatic pathologies.

16. Use according to Claim 15 for the treatment of diabetes.

- 17. A bioartificial pancreas, characterized in that it contains insulin secreting cells which can be obtained by the process according to any one of Claims 1 to 13, cultured in a matrix.
- 18. A bioartificial pancreas, characterized in that it contains insulin secreting cells which can be obtained by the process according to any one of Claims 1 to 13, cultured in a matrix.